REMARKS

Claims 21-40 are pending in the instant application. Claims 24, 26, 28, 37 and 38 are cancelled and claims 21 and 22 are amended. Upon entry of these amendments, claims 21-23, 25, 27, 29-36, 39, and 40 will be pending in this application.

Applicants note the withdrawal of the rejection of claims 1-20.

Rejection of claims under 35 U.S.C. §112, first paragraph, enablement

Claims 21-40 were rejected as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention. In particular, the Examiner stated on page 5, paragraph 2, of the Office Action that the specification does not provide any substantive examples demonstrating that the cells of the invention will maintain a precursor like state or will differentiate into mature hepatocytes *in vitro* or *in vivo*. Applicants respectfully traverse the Examiner's rejection in part.

The ability of liver precursor cells to differentiate is amply shown by papers in the field of the invention. Sigal et al. (1995) (a copy of which is attached hereto), transplanted precursor cells that expressed dipeptidyl peptidase IV (DPPIV) into rats that lacked this cell marker, and found expression of the marker in hepatocytes in the recipient animals. "There were numerous epithelial cells . . . with a morphology similar to adult hepatocytes. Transplanted cells were unequivocally localized by the DPPIV positivity." Page 41, paragraph four. Dabeva et al. (2000) (a copy of which is attached hereto) reach the same result and state that "[o]ne week after transplantation of FLEC [fetal liver epithelial cells] into the liver of normal adult rats, cells scattered throughout the parenchyma were diffusely stained for DPPIV... This suggested that the cells were not fully differentiated. Two weeks after transplantation, cells in the parenchyma . . . acquired an hepatocytic morphology with canalicular expression of DPPIV." Page 2022, paragraph one.

Furthermore, the Examiner stated that the specification does not demonstrate that one can genetically manipulate said cells *in vitro*, and that the specification "provides a curt description of methodology for inserting a gene of interest and administering said cell for treatment." Manipulation of cells is conventional once Applicants have taught how hepatocyte precursors are

obtained. The art is replete with examples of *in vitro* manipulation of cells, which are then administered to a patient in need thereof.

For example, Culver et al. (1991) (a copy of which is attached hereto and which is also cited in the accompanying Information Disclosure Statement), demonstrated that human leukocytes could be transfected *in vitro* with the human gene for adenosine deaminase (ADA) using a retroviral vector. Id. at page 108, paragraph 3. The autologous, genetically engineered cells were then infused into ADA-deficient children. Id. at page 108, paragraph 4. "Neither child has demonstrated significant side effects resulting from the infusion. . . [T]he first child *now* is making normal amounts of isohemagglutinins." Id. (emphasis in the original.)

Onodera et al. (1998) (a copy of which is attached hereto) reported successful gene therapy of a 5 year old boy with severe combined immune deficiency caused by ADA deficiency. These authors used LASN retrovirus to transfect autologous peripheral T-lymphocytes with the human ADA gene. Id., page 31, paragraph 2, and page 32 paragraph 1. "No selection procedure to enrich for gene-transduced cells was performed." Id. "ADA enzyme activity, nearly undetectable in the patient's lymphocytes before gene therapy, . . . reached 27 U on protocol day 476, which is approximately comparable to that a heterozygous carrier individual (the patient's mother, 34.8 U)." Id. at page 32, paragraph 2. "Both trials have shown *high gene transfer efficiency, remarkable increase of the ADA enzyme activity* and eventual improvement of the immune function." Id. at page 34, paragraph 0, (emphasis added). "[He] has gained 3 kg in weight . . . and is attending public school." Id. at page 33 paragraph 3.

Cavazzana-Calvo et al. (2000) (a copy of the abstract of which is attached hereto), reported gene therapy of an 11 month-old and an 8 month-old suffering from severe combined immunodeficiency-X1 disease caused by gamma-c cytokine receptor deficiency that leads to an early block in T and NK cell development. The marrow of the patients was harvested, $CD34^+$ cells selected and transfected. Id. at 669, paragraph 2. The transfected $CD34^+$ cells "were infused without prior chemoablation." Id. "Subsequently, T cell counts . . . reached values of ~2800/ul after 8 months." Id. "After primary vaccination, in vitro T cell proliferative responses . . . were observed within normal range." Id. at page 670, paragraph 0.

Applicants seek merely to apply standard methods of cell transfection and administration using the novel isolated hepatocyte precursors of the invention.

The Examiner evaluated the state of the art by referring to two recent review articles, one by Verma, et al. and one by Anderson, both of which suggest that difficulties remain with (1) efficient delivery of genes and (2) sustaining gene expression.

Applicants respectfully assert that efficiency of gene delivery is not an issue as Applicants are claiming transfection of precursor cells followed by transplanting the transfected precursors and/or their progeny. Cells bearing the gene of interest can be selected based on cotransfection with a selection marker, a conventional procedure. *See* page 9, line 1 of the specification. Moreover, transfection with even a few copies of the relevant gene can be effective. Thus, delivery of the genes is reduced to the much simpler problem of delivery of the cells, which can be accomplished by standard techniques, including surgery, as disclosed on page 12, line 6, for example.

Applicants also assert that sustaining gene expression is adequately addressed in the specification. For example, on page 11, line 5, states that "a construct in which there is an additional promoter modulated by an external factor of signal can be used, *making it possible to control the level of polypeptide* being produced by the modified hepatocyte precursors, or by mature hepatocytes which have differentiated from such precursors, by providing that external factor or signal" (emphasis added). On page 12, line 23, the Applicants disclose that "[o]nce in the liver [genetically engineered hepatocyte precursors] may express the gene(s) of interest and/or differentiate into mature hepatocytes which express the gene(s) of interest." In addition, the specification teaches on page 12, line 7, that genetically engineered hepatocyte precursors or a portion of their progeny differentiates into mature hepatocytes, which *provide a continuous supply of the protein*, polypeptide, hormone, enzyme, or drug *encoded by the gene(s) of interest*" (emphasis added). As the articles discussed above demonstrate, similar approaches are recorded in the literature as providing some notable successes.

Finally, the Examiner also stated, on page 8, line 8, of the Office Action, that the successful transfer of cells and tissues from one species to another continues to pose several technical difficulties due to immunological barriers. In contrast, the Examiner stated that "transplantation of tissues from one individual to another who demonstrates a tolerance due to histocompatibility is generally accepted in the art." Page 8, line 8, of the Office Action.

Claim 21 has been amended in this regard to recite the use of autologous cells. Support for this amendment is found on page 10, line 10. Claim 22 has been amended in this regard to

claim the transfer of a genetically engineered human hepatocyte precursor, progeny thereof, or both from one human to another human. Support for the language of the claim is found as cited above and on page 8, line 20.

Reconsideration is respectfully requested.

Rejection of claims under 35 U.S.C. §112, first paragraph, new matter

The Examiner rejected claims 22 and 24 as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the invention was filed, had possession of the claimed invention. In particular, the Examiner objected to the phrase 'with the proviso that xenogeneic administration is excluded' in claim 22 and 'autologous' in claim 24.

Applicants assert that the subject matter of claims 22 and 24 is adequately disclosed in the specification. However, without acquiescing to the propriety of the Examiner's rejection and solely to advance prosecution, claims 21 (which incorporates the subject matter of claim 24, which has been cancelled herein) and 22 and have been amended. Literal support for the amended claims, including the use of the term 'autologous' can be found in the present specification, as specified below.

The language of claim 21 is found in the specification as stated above. Support for the language of claim 22, as amended, is found as mentioned above and in the specification on page 9, line 26, which discloses that the genetically engineered cells of the invention "may be employed in treating any disease which results from a single gene defect which can be corrected by expression of the normal gene in the hepatocyte precursors or the differentiated cells derived therefrom." Moreover, the specification, on page 9 line 18, teaches "hepatocyte precursors to be genetically modified *ex vivo* can be obtained from a human." Later in the same sentence, on page 9, line 21, the text states that hepatocyte precursors "can be obtained from a donor (i.e., a source other than the ultimate recipient), modified and placed into a recipient, again by transplanting or grafting." On page 9, line 34, the specification states that the genetically engineered hepatocyte precursors "can be used to correct an inherited" disease.

Reconsideration is respectfully requested.

Rejection under 35 U.S.C. §112, second paragraph

The Examiner rejected claims 21-39 for being unclear and incomplete because they recite a method of treatment of liver dysfunction but do not recite steps where treatment is affected, only steps of administration.

As amended, claims 21 and 22 and the claims which depend therefrom, claim a method of treating liver dysfunction comprising the administration of genetically engineered hepatocyte precursors and treating the liver dysfunction.

The Examiner rejected claims 21-40 for being indefinite for using the term "hepatocyte precursors." This rejection is respectfully traversed.

Applicants maintain that the meaning of hepatocyte precursors is abundantly clear from the specification and would not be ambiguous to one of skill in the art based on the instant disclosure of the invention. Applicants respectfully draw the attention of the Examiner to page 1, line 21: "Such cells are sometimes hereinafter referred to as "hepatocyte precursors."

The Examiner rejected claims 24, 26, 28, 37 and 38 for being indefinite. Without acquiescing to the propriety of the Examiner's rejections with respect to these claims and solely to advance prosecution, claims 24, 26, 28, 37, and 38 have been cancelled.

The Examiner rejected claims 29 for lack of clarity in reciting "expresses at least one gene of interest" and claims 30 to 33 for reciting "the gene of interest." The rejection is respectfully traversed.

Applicant respectfully draws the attention of the Examiner to the extensive disclosure on page 8, line 14, et seq.:

> Gene(s) of interest which may be expressed by the hepatocyte precursors, or the differentiated cells derived therefrom, include, but are not limited to: (1) gene(s) present in and expressed at biologically effective levels by normal liver cells, but present in and expressed in less than normal quantities in the liver cells of animals or human patients to be treated prior to transfer of gene(s) of interest into them; (2) gene(s) not expressed in normal mature liver cells; or (3) gene(s) expressed in normal mature liver cells but whose structure is defective in the animals or patients to be treated, leading to the production of a non-functional protein, alone, or in any combination thereof.

Moreover, on page 12, line 28, the specification amplifies the language used by reciting: "the genetically engineered hepatocyte precursors or a portion of their progeny differentiates into mature hepatocytes, which provide a continuous supply of the protein, polypeptide, hormone, enzyme, or drug encoded by the gene(s) of interest." Applicants maintain that the claims are broad but not unclear.

The Examiner rejected claim 40 for being indefinite in the recitation of a 'drug delivery system' and in the recitation of 'in a biologically significant amount.' This rejection is respectfully traversed.

The language of claim 40, drawn to a drug delivery system, is supported by the language of the specification on page 13, line 7, which discloses that "[t]he hepatocyte precursors of the mature hepatocyte progeny therefrom, formed in this [genetically engineered] way can serve as a continuous drug delivery system to replace present regimens." Page 13, line 4, provides an understanding of "this way" by stating that "the hepatocyte precursors are genetically engineered in such a manner that they produce a gene product (e.g., a polypeptide or a protein) of interest in biologically significant amounts." These disclosures are supplemented by the disclosure on page 9, line 29, to the effect that "[g]enetically engineered cells of the present invention may be used, for example, for the delivery of polypeptides or proteins which are useful in prevention and therapy of an acquired or inherited defect." Moreover, on page 8, line 17, the specification teaches that the gene of interest is "expressed at biologically effective levels." Thus, as the claim is clearly supported in the language of the specification.

Reconsideration is respectfully requested.

CONCLUSION

It is believed that all of the stated grounds of objection and rejection have been properly traversed, overcome, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn because they cannot be sustained. Applicants believe that the present application is in condition for allowance.

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned directly.

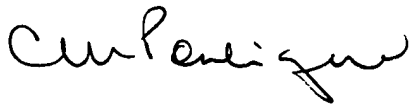
Prompt and favorable consideration of this Response is respectfully requested.

AUTHORIZATION

The Commissioner is hereby authorized to charge any additional fees, including fees for the net addition of claims, which may be required for this Response, or credit any overpayment to Deposit Account No. 50-0436.

In the event that an Extension of Time is required, or which may be required, in addition to that requested in a petition for an Extension of Time, the Commissioner is requested to grant a petition for that Extension of Time which is required to make this response timely and is hereby authorized to charge any fee for such an Extension of Time or credit any overpayment for such an Extension of Time to Deposit Account No. 50-0436.

Respectfully submitted,
PEPPER HAMILTON LLP



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APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Amended claims, to show changes:

21. (Amended) A method of treatment of liver dysfunction in a subject in need thereof comprising administering a genetically engineered autologous hepatocyte precursor, progeny thereof, or both, to the subject and treating liver dysfunction.

22. (Amended) A method of treatment of liver dysfunction in a human subject in need thereof comprising administering a genetically engineered human hepatocyte precursor, progeny thereof, or both, to the human subject[, with the proviso that xenogenic administration is excluded] subject and treating liver dysfunction.